# UNIVERSITY DEPARTMENT OF ZOOLOGY

# RANCHI UNIVERSITY, RANCHI

**SYNOPSIS FOR Ph.D.**

**TOPIC**

Effect of *Centella asiatica* (Beng saag) in the gut of *Rattus norvegicus* (Albino Wistar rats) induced with irritable bowel syndrome.


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# State of the art.

## Introduction.

Irritable bowel syndrome is a multi-symptomatic and multifactorial disease where an individual is sensitive to almost all kinds of foods. Any food taken in leads to discomfort and gut irritability.

### Current knowledge.

'Lekha Saha' (2014) reported that historically, IBS is known for over 150 years. 'Cumming' (in 1849) reported the bowels are one time constipated, another lax in the same person.

Recently 'Hana Ames' (2024) talks about mainly three types of IBS:

**IBS with Constipation** (IBS-C).

National Institute of Diabetes and Digestive and Kidney Diseases reported that a person has at least one abnormal bowel movement where more than ¼ of their stools will be hard and lumpy and less than ¼ loose and watery.

**Common symptoms are:**

- Abdominal cramps and distress.
- Chronic Constipation.
- Difficulty in stool passing.
- Bloating and gas.
- Mucus in stool.
- Incomplete defecation.

**IBS with diarrhea** (IBS D).

It is related to chronic diarrhea and common symptoms include:

- Chronic diarrhea.
- Abdominal cramps.
- Bloating and gas.
- Urgency.
- Increase bowel movements.
- Mucus in stool.

**IBS mixed** (IBS-M).

This is related to alternating between Constipation and diarrhea and involves the same symptoms mentioned above.

Some other types also include post-infectious IBS or PI-IBS (that is, after interaction with some infection that leads to gastroenteritis) and post-diverticulitis IBS. These are explained and reported by 'Erica et al.' (2013). This report states that a person with diverticulitis may have an increased risk of IBS.

'Riddle et al. (2016) in an investigation of risk factors for military personnel developing IBS, learned that the risk of developing IBS increases after cases of some infectious gastroenteritis, post-traumatic stress disorder, increased number of life stressors, self-reported anxiety, and depression.

All this knowledge about IBS explains the brain's and gut's interdependence, a relation often known as brain- gut axis or BGA.

'Ohman et al. (2007) state that BGA is a pathway among physiological systems, which include the central nervous system, enteric nervous system, and autonomic nervous system.

## Context of the research problem.

Even after several advancements in understanding and treatment of this multi-symptomatic disease, there still exists several gaps and challenges in treating and managing IBS.

'Camilleri' (2014) reported that despite its frequency, the complete pathophysiology of IBS is not completely understood. Many factors like gut microbiota imbalance, altered brain-gut communication, and visceral hypersensitivity are thought to play some role

The unique symptom profile of this disease makes the treatment more challenging.

**Adapted therapeutic approach.**

'Ford et al.'(2017) reported that the manifestation of IBS is different in a different person. One treatment may not be helpful in every case of IBS. It has to be dependent on the adaptive features.

**Limited effective medication**.

'Staudacher et al.'(2017) reported that medications are available for this multifactorial disease, which include laxatives, antispasmodics, and anti-

diarrheal whose effectiveness can vary. For some, medications including 'Rifaximin' and 'Lubiprostone' may help. However, to date, there is no only and only one effective solution that can cure all symptoms of IBS.

**Understanding of pathophysiology**.

'Camilleri, M' (2018) reported that the main reason for the cause of IBS is not completely understood. It is thought to involve visceral hypersensitivity, gastrointestinal motility issues, and malfunctioning of the brain-gut regulatory system. Therefore, in order to understand these mechanisms efficiently, more research is required.

**No universally accepted diagnostic test.**

'Moayyedi' et. al (2014) states that since it is a multi-factorial and multi-symptomatic disease, there is no universally accepted diagnostic test.

Due to the lack of efficient diagnostic tests for IBS, many times misdiagnosis or delays in treatment can take place.

The complexity of IBS is no doubt highlighted through current research, but treatment approaches remain suboptimal, which completely focus on the management of symptoms rather than addressing the core causes.

This uncertainty emphasized the requirement for further exploration into the mechanism driving IBS and the development of more targeted, effective therapies.

One way to address the core causes is by using specific plant extracts, an area that is not completely explored for the treatment of IBS.

**The present research is directed towards finding out how effectively IBS could be managed in the long term and the problem of symptoms reoccurrence and changes in symptoms of IBS could be solved by the use of plant extract of *Centella asiatica.***

**Why *Centella asiatica*?**

Much of the research highlights the positive effect of *Centella asiatica* in the gut. Even in Ayurveda, this plant is mentioned as a Rasayana that is a medicine for various abdominal disorders reported by 'K Sairam' et al. (2000).

'Olabisi et al.' (2023) reported that the use of *Centella asiatica* improves the overall health of the birds and improves beneficial bacteria in the gastrointestinal tract.

'Vasantharuba' et al. (2012) reported the Antioxidant, Antibacterial, Antifungal, Antiviral, Anti-protozoal, anti-filarial, anti-ulcer, and anti-inflammatory activity of *Centella asiatica***.**

'M.A. Abdulla et al.' (2010) reported that '*Centella asiatica* leaf extract exhibited significant protection against gastric mucosal injury in rats'

## Problems to be investigated.

1. Investigation of gut microbiota in controlled Albino Wistar rats.
2. Effect of *Centella asiatica* in the gut of Albino Wistar rats induced with IBS.
3. Histological and biochemical assessment of the gut of Albino Wistar rats treated with IBS.

## Objectives.

1. To study the gut microbiota of controlled Albino Wistar rat.
2. To see the role of plant extract *Centella asiatica* in the Albino Wistar rats' gut which are induced with IBS.
3. To analyze and assess histology and biochemical changes in the gut of Albino Wistar rats (*Rattus norvegicus*) treated with IBS.

## Motivation.

*Centella asiatica* extract could help manage IBS in the long term and solve the problem of symptom reoccurrence.

## Hypothesis.

*Centella Asiatica* (Beng Saag), an indigenous plant species of Jharkhand, may prove to be impactful and effective in treating IBS.

It is hypothesized that leaf extract of *Centella asiatica* may help in solving the problem of symptom reoccurrence and frequent changes in symptoms associated with managing IBS in the long term.

## Research design and methodology.

**Collection and preparation of *Centella asiatica* leaf extract**.

Fresh leaves of *Centella asiatica* will be collected from local vegetable vendors, washed thoroughly, and shade-dried for 7 to 10 days to preserve bioactive compounds. Leaves will be boiled in distilled water under reflux for 1 hour. To obtain powder the liquid is first filtered and then freeze-dried, before use, the dried powder will be re-dissolved in distilled water (Gou et al. 2004).

**Procurement of rats**.

For this study, 24 albino Wistar rats were selected due to their physiological relevance to the proposed research on the effect of *Centella asiatica* on the gut of IBS-infected mammals. These rats will be selected based on their specific genetic background and health status, which are aligned with the study's objective.

All animals will come with documentation verifying their genetic background and pathogen-free status.

The study will be carried out only after being approved by the Institutional Animal Care and Use Committee (IACUC) to compile with ethical guidelines and it will follow the 3R principle (Rushell et al. 1959).

**Acclimatization of rats**

Acclimatization of albino Wistar rats will be conducted over a 5-7 days period (Institute of Laboratory Animal Research, 2011), during which the animal will be housed in controlled environmental conditions, including a temperature of 20 to 24°C, humidity of 45 to 55% and 12 hour light/dark cycle (Council of Europe for Vertebrate Protection, 2006).

This allows the animal to adjust to their new surroundings, minimizing stress and stabilizing physiological and behavioral parameters (Balcombe et al. 2004). The rats will be provided with ad-libitum access to food and water and their intake will be regularly monitored. Thus, the acclimatization phase is essential to ensure the validity and reproducibility of experimental results.

**Control and experimental groups.**

Rats will be divided into the following groups:

- **Control group:** Healthy rats with no IBS induction.
- **IBS-induced group:** Rats induced with IBS but receiving no treatment.
- **Treatment Group 1**: IBS-induced rats treated with 500 mg per kilogram body weight of *Centella asiatica* extract.

- **Treatment group 2**: IBS-induced rats treated with 1000 mg per kilogram body weight of *Centella asiatica*.

**Induction of IBS.**

IBS will be induced in the rats using a 4% acetic acid solution prepared by diluting glacial acetic acid with saline (Tian et. al,2017). 1-2ml of 4% acetic acid solution will be administered intra-rectally using a catheter. 2-3 days after administration, the rats usually show signs of IBS, such as visceral hypersensitivity and altered bowel habits (Al-chaer et. al 2004) (Zhou et al. 2009) (Ohman et al. 2010).

**Treatment with *Centella asiatica***.

Following IBS induction, treatment groups will receive *Centella asiatica* orally using a gastric gavage tube, at a dose of 500 mg per kilogram and 1000 mg per kilogram of body weight for four weeks twice daily (K Sairam et al. 2000) (Sudha et. al 2002)

**Parameters for assessment**.

Physiological assessment will include body weight, stool consistency, and general appearance of the rat (grimace) (Ghattamaneni et al. 2018).

Abdominal withdrawal reflex will be seen if there is visceral hypersensitivity and histological analysis to assess intestinal inflammation (Yu An et al. 2009)

**Statistical Analysis.**

Data will be statistically analysed using ANOVA and T-test with a significant set of $P<0.05$.

## Tools to be used.

- Animal housing: Cages, Bedding, temperature/humidity control.
- IBS induction: Inducing agent, syringes/needles.
- Oral gavage equipment, precision scales.
- Body weight scales, stool collection, and analysis tool.
- Biochemical assays: ELISA Reader, spectrophotometer, centrifuges, pipette.
- Histological analysis tool, Microtome, embedding station, Histology staining kits.
- Microscope: Light or electron microscope.
- Vortex mixture and shaker.

- Refrigerators and freezers.

## Significance of study.

- The study will be helpful in better understanding IBS, its cause, and symptoms.
- It will help in solving the problem of symptoms reoccurrence in IBS.
- Information will be used in the management of disease in a sustainable tune.
- Information will be helpful in the long-term management of IBS.

## Future aspects and contribution to the society.

The future aspect of this present work is to explore the molecular mechanism through which *Centella asiatica* affects gut health. Researchers could find new treatments or supplements for IBS based on *Centella asiatica.*

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